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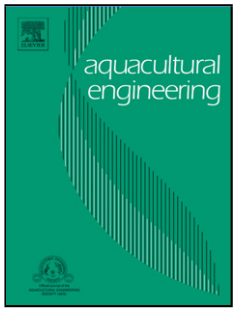
Pedersen, Lars-Flemming; Pedersen, Per Bovbjerg

Published in:
Aquacultural Engineering

Link to article, DOI:
10.1016/j.aquaeng.2011.11.001

Publication date:
2012



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Authors: Lars-Flemming Pedersen, Per B. Pedersen

PII: S0144-8609(11)00079-3
DOI: doi:10.1016/j.aquaeng.2011.11.001
Reference: AQUE 1610

To appear in: *Aquacultural Engineering*

Received date: 16-8-2011
Revised date: 1-11-2011
Accepted date: 9-11-2011

Please cite this article as: Pedersen, L.-F., Pedersen, P.B., Hydrogen peroxide application to a, commercial recirculating aquaculture system, *Aquacultural Engineering* (2010), doi:10.1016/j.aquaeng.2011.11.001

- Full scale test and application of H_2O_2 on a commercial model trout farm
- Step-by-step approach including characterization of biofilter nitrification capacity before and after H_2O_2 application (analytically verified)
- Beneficial environmental and hygiene aspects of the reported H_2O_2 application

Hydrogen peroxide application to a commercial recirculating aquaculture system

Lars-Flemming Pedersen[*1] and Per B. Pedersen[1].

[1] Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark.

Running title: "*Hydrogen peroxide application to commercial RAS*"

Hydrogen peroxide application to a commercial recirculating aquaculture system

Abstract.
An important part of the management of recirculating aquacultural systems is to ensure proper rearing conditions in terms of optimal water quality. Besides biofiltration, current methods include use of use of micro-screens, UV irradiance and use of various chemical therapeutics and water borne disinfectants. Here we present a low dose hydrogen peroxide (H_2O_2) water hygiene practice tested on a commercial Model Trout Farm. The study included application of H_2O_2 in a separate biofilter section and in the raceways with trout. Peroxide addition to the biofilter (C_0=64 mg H_2O_2/L) significantly reduced ammonium removal efficiency (0.13 vs. 0.60 g $N \cdot m^{-2} \cdot d^{-1}$) and nitrification partly recuperated within 7 days. Nitrite removal after H_2O_2 addition was only slightly impaired and no build-up of either ammonia/ammonium or nitrite was observed in the system. Application of H_2O_2 was rapidly degraded and caused substantial release of organic matter from the biofilter and hence increased the water flow and improved the hydraulic distribution through the biofilter. Low concentration H_2O_2 of about 15 mg/L was obtained in the raceways for three hours with temporarily disconnected biofilter sections, until H_2O_2 levels were < 5 mg/L and considered safe to re-introduce to the biofilter sections. H_2O_2 addition in the raceways appeared to improve the water quality and did not affect the fish negatively. The study illustrates the options of using an environmental benign, easily degradable disinfectant and challenge the dogma that hydrogen peroxide is not suitable to recirculating aquaculture systems due to the risk of a biofilter collapse.

Key words: management practice, water quality, hygiene, disinfection, biofilter nitrification, model trout farm, environmental impact

I. INTRODUCTION

In order to achieve proper fish rearing conditions, the occasional use of chemical disinfectants such as formalin, copper sulphate, Chloramine-T, peracetic acid, or hydrogen peroxide are commonly used (Boyd and Massaut, 1999, Rintimäkki et al., 2005). The applications range from egg disinfection (Wagner et al., 2008) to system sanitization (Waldrop et al., 2009) and are often used to control fungal and bacterial growth and to suppress parasitic load in systems where preventive biosecurity measures are insufficient (Rach et al., 2000; Schmidt et al., 2006; Kristensen & Buchman 2009).

Numerous considerations must be made when administering disinfection treatments. For example, a high treatment efficacy against the target organisms has to be achieved while fish health, food , worker and environmental safety are not compromised. An additional concern that relates to recirculating aquaculture systems (RAS) is the risk of impairing communities of nitrifying bacteriain the biofilters, potentially causing substantial ammonia and/or nitrite accumulation (Noble and Summerfelt, 1996; Pedersen et al, 2009).

Pressure from external parasites can be controlled, either preventively or curatively, by regular water treatment practices over a prolonged period of time by applying either formalin or sodium chloride or a combination thereof (Mifsud & Rowland, 2008). Both agents can suppress pathogen levels and decease fish mortality (N.H. Henriksen, Danish Aquaculture Organisation, pers. Comm) but the treatment regimens used have drawbacks, which leaves room for further improvement. Beside a worker safety issue (Lee and Radtke, 1998), formalin in systems with short retention time and without biofilters can potentially result in a concomitant discharge of formaldehyde exceeding the values set by national authorities (The Environmental Protection Agency under Danish Ministry of the Environment (Pedersen et al, 2007). Sodium chloride is typically applied to raise the salinity to 5-15 ‰ which require substantial amounts of salt (5-15 kg per m^3), potentially impacting the receiving water body. Non-chemical mechanical control (Shinn et al, 2009) or UV irradiation (Sharrer et al, 2005) are other options that have been documented to control important parasite infections, but these measures are presently not economically feasible to the majority of commercial, outdoor aquaculture operations.

Hydrogen peroxide (H_2O_2) fulfills the requirements asan alternative candidate for aquaculture disinfection (Schmidt et al., 2006), and is an example of an environmentally benign chemical (Block, 2001). Hydrogen peroxide is easily degradable and does not create harmful disinfection by-products and hence, it is not expected to cause environmental concerns. Hydrogen peroxide complies with most principles of green chemistry, defined as "the utilisation of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products" (Anastas & Warner, 1998). Nevertheless, formalin is still a preferred chemical, and in order to change common practice, further documentation on the safety and efficacy of H_2O_2 is therefore needed.

Different studies have focused on various aspects of H_2O_2 application in aquaculture (reviewed in Schmidt et al., 2006). Treatment efficacy studies with H_2O_2 have been reported (e.g. Rach et al., 1997; Gaikowski et al., 2000) as well as analytical verification of

H_2O_2 concentration during treatment (Rach et al., 1997; Rach & Ramsey, 2000, Pedersen et al., 2011) environmental issues (Saez and Bowser, 2001) and studies related to H_2O_2 application in aquaculture systems with biofilters (Schwartz et al., 2000, Møller et al., 2010, Pedersen et al., 2011).

Heinecke & Buchmann (2009) documented the antiparasitic effects of the H_2O_2 releasing compound sodium percarbonate against *Ichthyophthirius. multifiliis* in a laboratory study. These dose-response correlations allow aquaculturists to adapt their own system-specific water treatment routines. In case of implementing prolonged low dose H_2O_2 [≤ 15 mg/L H2O2) exposure it has to be considered thought that the laboratory data was obtain under conditions not directly comparable to practical farming operation. To implement this lab-based suggestion, effective on-farm treatment regimens have to be practical and realistic. Therefore, reliable sets of guidelines tested at real farming conditions are needed to accelerate the generation of a new, alternative water treatment management practice.

The goal of this study was to investigate the potential of H_2O_2 as a viable water treatment procedure in a commercial,freshwater trout farm. The study mimicked water treatment regimens in full scale, by including analytical verification of H_2O_2 concentrations and an assessment of the potential impairment of the nitrifying activity in the biofilters. Issues of water treatment management practice, present limitations and future perspectives are presented and discussed.

2. MATERIALS AND METHODS

2.1. *Description of aquaculture facility*

The experiments were carried out at Tingkærvad Dambrug (Randbøldal, Denmark), a commercial freshwater recirculating aquaculture system. The particular aquaculture system (Model Troutfarm concept) consisted of 12 interconnected raceways (each 150 m^3), four airlifts, two side-blowers, a 70 µm drum filter and a biofilter section consisting of 6 separate biofilters in parallel (Fig. 1; Table 1). Make up water (groundwater) was approximately 20 l/s with an internal flow of 600 l/s (velocity 10 cm/s) circulated by 4 airlifts each connected to a side-blower. The farm produced rainbow trout Oncorhynchus *mykiss* (250-400g) and had an approximate standing stock ranging from 30 to 35 metric tonnes during experiments. Fish feed (Biomar, Denmark) equivalent to approximately 1 % body mass/day were administered during the period from 6 a.m. to 6 p.m.

Three separate experiments were sequentially carried out at the trout farm during a summer period: i) High dose single point H_2O_2 addition to a closed biofilter section, ii) Single point H_2O_2 addition to the raceways, and iii) Multiple H_2O_2 addition to the raceways and evaluation of associated biofilter performance.

2.2. *Experiment I: High dose single point H_2O_2 addition to a closed biofilter section*

Two identical biofilter sections were randomly selected s for this experiment. One biofilter section was acutely exposed to H_2O_2. In connection with H_2O_2 application, water inlet to the test biofilter section was shortly sealed off as a common management routine and to avoid any leakage. From this biofilter section duplicate samples of biofilter elements were collected just prior to H_2O_2 exposure and at three other occasions (1 hr., 18 hrs. and 7 days aftert exposure) A neighbouring biofilter sectionserved as a control and biofilter elements not exposed to H_2O_2were samples as control.

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The H_2O_2 exposed biofilter section was fitted with Hach Lange online sensors (pH, $_{Redox}$, $_{Oxygen}$, and conductivity) connected to HQ40D multimeters® (Hach Lange, Loveland, Co.USA) to monitor potential changes related to H_2O_2 addition and degradation. A total of 10 kg 35 w/w % H_2O_2, equivalent to 3500 g H_2O_2, with a nominal H_2O_2 concentration equivalent to 64 mg/L was added and distributed evenly to the test biofilter section, and water samples were collected and fixed at regular intervals. Biofilter performances were evaluated in terms of standardised ammonia/ammonium and nitrite spiking experiments with representative subsamples of biofilter elements. Biofilter elements of equal volume (0.90 l) were transferred (duplicate subsampling and performance test) to aerated batch reactors and each supplied with 2.3 liter system water (Møller et al, 2010). After 0.5 hours of acclimatization, stock solutions of either NH_4Cl or $NaNO_2$ were added. Water samples were collected and filtered (0.2 µm Sartorius®) every 5 minutes until almost complete N-oxidation was achieved.

2.3. *Experiment II: Single point H_2O_2 addition to raceways*

This experiment was a preliminary test to investigate distribution and hydraulic patterns as well as to determine the magnitude of H_2O_2 degradation rate. A total of 20 L of 35 % H_2O_2 was quickly added to the airlift located at the inlet to rearing section 1 (Fig. 1). Based on predicted mixing and water velocity as well as the fish behaviour in front of the H_2O_2 pulse, different consecutive sampling locations were identified for collecting water samples for the analytical verification of H_2O_2 concentration. Each section was 25 meter long, resulting in a total linear distance of 300 meter from biofilter outlet to inlet.. Concurrently, the farm manager used H_2O_2 sticks (Merckoquant® 110011 [range:0-25 mg/L H_2O_2) to follow the chemical pulse and to ensure that corresponding actions could be taken in a timely manner, in case H_2O_2 concentration level became critical for the biological filters. As a precautionary action bulkheads were removed between ends of raceways, thereby bypassing the biofilters (Fig.1)

2.4. *Experiment III: Multiple and prolonged H_2O_2 addition to the raceways and evaluation of implications on biofilter activity*

The purpose of this experiment was to test a H_2O_2 treatment regimen averaging10 mg H_2O_2 /L for 3 hours, based on Henicke and Buchmann (2009) and recommended by veterinarian (N. H. Henriksen, Danish Aquaculture Association, pers. comm.). Prior to the application, the entire biofilter (all 6 sections) was bypassed by removing wood bulkheads in the

raceway sections and aeration was ceased in the biofilter sections to minimize water flow into the biofilter sections. Doing this, water was redirected from raceway 6 and 12 back to raceway 1 and 7, respectively, creating two closed recirculation loops (as shown in Fig. 1). Representative subsamples of biofilter elements were collected from a biofilter sections and served as a control for the baseline nitrification performance.

The total application of H_2O_2 was 80 litre 35% H_2O_2, equivalent to c. 31.6 kg H_2O_2 with a theoretical nominal concentration around 20 mg H_2O_2/L in the rearing units. To ensure ideal mixing and an even distribution of H_2O_2, 20 liter of H_2O_2 were concurrently added into each of the four airlifts. Unlike Experiment 2, H_2O_2 was added over a prolonged period of time of 15 minutes, corresponding to the theoretical retention time in the four rearing units, by use of 25 liter barrels with a 5 mm hole at the bottom. Water samples were collected at the outlet of raceway 6 and 12 during the experiment. Three hours after to experimental commencement, it was decided to reopen the biofilter flow to two of the six biofilter sections, as H_2O_2 concentration was sufficiently low (< 5 mg H_2O_2/L according to sticks). Forty-five minutes later, all biofilters were in normal operation.

Similar to Experiment I, biofilter nitrification performance of unexposed and H_2O_2 exposed biofilter elements were evaluated in bench scale reactors with NH_4Cl spiking. Three samples of biofilter elements were tested: control (prior to H_2O_2 exposure); minimally exposed (three hours after H_2O_2 exposure and by-passed from the raceway); and biofilter elements exposed to residual H_2O_2 (sampled additional 45 minutes after reopening the biofilter, corresponding to 3¾ hours after H_2O_2 exposure in the raceway).

2.5. *Analysis*

Water samples for total ammonia/ammonium-nitrogen (TAN), nitrite-N and nitrate-N were analysed immediately, or kept refrigerated at 5° C for later analysis. Samples for determination of organic matter content as chemicical oxygen demand (COD) were fixed with 2 ml 4 M HCL /L sample and kept frozen for subsequent analysis. Chemical analysis of total ammonia/ammonium-N (TAN), nitrite-N and COD where made as described by Pedersen et al., 2009; H_2O_2 analysis were made according to Tanner and Wong (1998) modified by four-fold stronger fixating reagents, made with 1.2 g NH4VO3, 5.2 g dipicolinic acid and 60 ml conc. H2SO4.

3. RESULTS

3.1. *Single point H_2O_2 addition to a closed biofilter section*

The theoretical initial H_2O_2 concentration of 64 mg/L was reached shortly after addition, only to exponentially decrease to baseline during the following 30 minutes (Fig. 2). After mixing, H_2O_2 concentration decayed exponentially according to the equation $Ct = C_0 \cdot e^{-kt}$, (Ct being the concentration at time=t; C_0 the nominal concentration at time=0 and k the exponential reaction rate) with a half-life of ~ 5 minutes, The first three measurement of H_2O_2 in the biofilter (all above 45 mg/LH_2O_2 (Fig.2) might be underestimated and connected with a some analytical variation due to the high absorbance in undiluted water samples.
The H_2O_2 application in the closed biofilter section led to significant fluctuations of oxygen and redox, whereas pH and conductivity did not change (Fig. 3). After H_2O_2 application, oxygen concentration reached an increased plateau approximately 2.5 mg O_2/L higher than prior to H_2O_2 application, indicating an instant inhibition of heterotrophic bacteria and autotrophic nitrifying bacteria. In association with the H_2O_2 addition, the biofilter section was vigorously aerated (submerged nozzles) following the common backwash protocol; as a result, excessive amounts of organic matter were shed into the water phase and directed to the sludge compartment.

The H_2O_2 application significantly inhibited biofilter nitrification in terms of reduced ammonia oxidation rates. Baseline ammonia oxidation rates (0° order) of unexposed biofilter elements were measured to be 0.59 g N/m^2/d. Test of H_2O_2 exposed biofilter elements at three different recovery times revealed significantly reduced ammonia oxidation rates of 0.24 N/m^2/d (1 hr), 0.13 N/m^2/d (18 hrs.) and 0.31 N/m^2/d (7 days) (Fig. 4; Table 2).
Comparative measures of TAN removal in biofilters from a neighbouring biofilter section revealed a rate of 0.61 N/m^2/d. Nitrite oxidation performance was evaluated similarly, and was found to be only marginally negatively affected compared to unexposed groups (Fig. 5; Table 2). The H_2O_2 procedure caused liberation of organic matter from the biofilter elements (COD values in the biofilter section after H_2O_2 application was measured to approx. 800 mg O_2/L, more than a forty-fold increase compared to the raceway water COD) and reduced the hydraulic resistance through the biofilter section.

3.2. *Single point H_2O_2 addition to production unit*

The fate of H_2O_2 throughout the rearing units when added to the airlift system at the inlet is shown in Fig. 6. Sampling at various positions revealed the consequences of dilution and decomposition, in terms of flattened and extended concentration peaks. The results from sampling point 12 showed that a substantial quantity of H_2O_2 was still present at the rear end of the production unit just prior to the inlet to the biofilter sections. At rearing unit 9, approximately 85 % of the total added H_2O_2 was measured as a plug flow pulse.

3.3. *Multiple* H_2O_2 *addition in production unit and biofilter evaluation*

The precautionary setup that allowed bypassing of the biofilter sections led to two identical loops within the production unit. Figure 7 shows the resulting H_2O_2 concentration in these two loops during a time span of 4 hours. In both loops, the application procedure led to initial fluctuations in H_2O_2 concentration during the first hour after addition, after which a steady decay occurred. Continuous exponentially decomposition of H_2O_2 occurred throughout the monitoring period with an approximate rate constant k of 0.45/h corresponding to half-lives of 1.5 hours.

Evaluation of ammonia oxidation performance showed that the biofilter elements from the biofilter section (disconnected from the rearing units with H_2O_2 for three hours and then exposed to residual H_2O_2 for 45 minutes) had sligthly reduced TAN removal rates of 0.56 $gN/m^2/d$ compared to unexposed (control) biofilter elements with TAN removal rates of 0.69 g $N/m^2/d$ (Table 2).

3.4. *Associated management issues*

All three experiments combined normal aquaculture operational practices with new therapeutic measures. Addition of H_2O_2 directly to the biofilter caused considerable liberation of organic matter. This was controlled by enclosing the biofilter section and redirecting the COD-enriched water to the sludge compartment. The applications of H_2O_2 in Experiments II and III were similar to normal practice with formalin using a simple dosage regulation in terms of prolonged application using a barrel/reservoir with a hole. The visual response of the trout to the chemical treatment was an aggregation downstream of the concentration pulse.
This reaction was similar to reactions associated with formalin application, but much less pronounced compared to fish reaction when peracetic acid compounds are applied (Jens Grøn, Farm manager; Personal comm.). The safety measures of isolating the production units from the biofilter sections was not common practice but was possible due to the system design and associated with some extra effort (< half an hour). During the experiments, the fish farmer successively used Merckoquant H_2O_2 sticks around the production unit and was able to obtain very reliable readings when compared with values from the chemical analysis. This monitoring allowed the fish farmer to potentially adjust the H_2O_2 concentration and to notice when the H_2O_2 level was sufficiently low (H_2O_2 < 5 mg/L) to let the water pass through the biofilter again.

4. DISCUSSION

This step-by-step test of H_2O_2 in a commercial operation provides new information to the fish farmer on how to implement a safer and more environmentally friendly water treatment practice. The actions taken were found not to harm the fish, and - though not quantified - the farm manager reported reduced fish mortality and improved water quality afterwards. Additionally, the altered treatment protocol was easily adopted, and the concomitant sanitation of the biofilter section (moderate biofilm control) was found to improve the biofilter hydraulics by removing particulate organic matter and loosen immobilized biofilter elements. The potential effects of impaired nitrification could, in this particular case, be circumvented by an alternating hygiene routine, e.g. sanitizing one of the six biofilter sections every second week.

Despite obvious beneficial attributes of H_2O_2 and well-known effects in North American hatcheries (Schmidt et al, 2006), H_2O_2 still remains relatively unproven in outdoor semi-recirculating aquaculture systems. Instead, the use of and experience with formaldehyde exceed by far the use of H_2O_2. Until recently, there has been little incentive for farmers to replace formaldehyde (Pedersen 2007). Recent Danish certified organic aquaculture requirements obligate farmers seeking this certification to operate their fish farm without using formaldehyde despite its known broad therapeutic range to control most common or important parasites in commercial conditions. Formaldehyde is known to have a broad therapeutic range and a high treatment efficacy against most common/important parasites under commercial conditions, except at low temperature conditions
Hands-on experience of using H_2O_2 by fish farmers is presently being gained. Recent investigations with application of low dose H_2O_2 in commercial fish farms have documented the ability of low dose H_2O_2 in eliminating a number of parasites (Pedersen & Henriksen, 2011). However, low dose H_2O_2 apparently has a limited effect against gill amoeba and *Ichthyobodo necator* (Costia) infections. Therefore, more potent treatment regimens are required to replace formaldehyde for these infections.

Increasing the H_2O_2 dose could potentially have detrimental effects on biofilter performance as observed in the present Experiment I and as reported by Schwartz et al. (2000). The study by Schwartz et al. (2000) was conducted with quantities of H_2O_2 equivalent to 100 mg H_2O_2/L and they observed an 80% reduction in ammonium removal in a fluidized sand bed filter. Both nitrification processes can be affected (Hagopian and Riley, 1998), but in the present experiment primarily ammonia oxidation was impaired. The immediate reduction in TAN removal rate was more pronounced than the nitrite oxidation, which is in contrast to other studies (Pedersen et al, 2009). The 3-4 fold decrease in TAN removal rate after one week suggests that the nitrifiers were inhibited and partially able to recover, considering the doubling time of several days (Hagopian and Riley, 1998). The water temperature was approximately 16.5°C at the day of experimentation; at this temperature, a two- to three-fold faster H_2O_2 decay would be expected compared to situations with water temperature at 6°C due to microbial activity (*Unpubl. data*). The relative high water temperature (ranging from 16 to 18°C) the following week also affected the recuperation of the nitrifiers, which expectedly would be significantly slower during colder conditions.

Møller et al. (2010) and Pedersen et al. (in press) found that transient low-dose H_2O_2 did not affect the nitrification process substantially, when tested in a pilot scale RAS with low organic and nitrogenous loading and a thin biofilm. Measures could be taken to avoid any biofilter impairment when using H_2O_2. The present results combined with the recommendations provided by Heinecke & Buchmann (2009) opens up for the option of treating water with low concentration of H_2O_2 also in commercial RAS with nitrifying biofilters.

There are certain additional hygiene aspects regarding the use of H_2O_2. Besides antiparasitic abilities (Block, 2001), recent studies have also documented the potential of H_2O_2 in combination with UV to improve water quality and control geosmine and -2-methylisoborneol (Klausen & Grønborg, 2010). Hydrogen peroxide products (high dose technical H_2O_2 or sodium percarbonate) appear to be compatible candidates to hypochlorite (Waldrop et al., 2009), when disinfection practices have to be fully implemented to RAS; this possibility deserves further attention.

In conclusion, the present study challenges the current paradigm of H_2O_2 being incompatible with RAS due to the risk of biofilter collapse. It was possible to maintain and control low dose H_2O_2 concentrations in a large, full scale RAS in commercial operation. Though not quantified, water quality was reported improved following H_2O_2 application and empirical observations indicate that a number of parasites were efficiently eliminated. It still remains untested whether H_2O_2 application in full scale systems can fully replace the use of formaldehyde, as low dose H_2O_2 application presently seems insufficient to fully control gill amoeba and *I.necator* (Costia) infections.

Acknowledgement
This study was financed by the Danish Ministry of Food, Agriculture and Fisheries and the European Union through the European Fisheries Fund (EFF). Thanks to farm manager Jens H. Grøn (Green) for experimental involvement and recommendations throughout the trials. Thanks to Niels Henrik Henriksen (Danish Aquaculture Organization) and Christopher Good (Freshwater Institute, WV, USA) for providing valuable comments and to Brian Møller, Dorthe Frandsen and Ulla Sproegel (DTU Aqua, Section for Aquaculture, Hirtshals, Dk) for chemical analysis and technical support during field work. Finally, thanks to three anonymous reviewers for constructive comments.

REFERENCES

Block,S.S. 2001. Peroxygen compounds *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 5th ed. LWW, Philadelphia, Pa. ISBN 0-683-30740-1.

Boyd,C.E. and Massaut,L. 1999. Risks associated with the use of chemicals in pond aquaculture. Aquacultural Engineering **20**: 113-132.

Clay,J.W. and Eds.Tucker,C.S.&.H.J.A. 2008. The role of better management practices in environmental management. In Environmental best management practices for aquaculture. Blackwell Publishing, ISBN-13: 978-0-8138-2027-9.

Fish, F. 1932. The chemical disinfection of trout ponds. Transactions of the American Fisheries society, Vol. 63: 158-163.

Gaikowski,M.P., Rach,J.J., and Ramsay,R.T. 1999. Acute toxicity of hydrogen peroxide treatments to selected lifestages of cold-, cool-, and warmwater fish. Aquaculture **178**: 191-207.

Hagopian,D.S. and Riley,J.G. 1998. A closer look at the bacteriology of nitrification. Aquacultural Engineering **18**: 223-244.

Heinecke,R.D. and Buchmann,K. 2009. Control of Ichthyophthirius multifiliis using a combination of water filtration and sodium percarbonate: Dose-response studies. Aquaculture **288**: 32-35.

Hohreiter,D.W. and Rigg,D.K. 2001. Derivation of ambient water quality criteria for formaldehyde. Chemosphere **45**: 471-486.

Jørgensen,T.R., Larsen,T.B., and Buchmann,K. 2009. Parasite infections in recirculated rainbow trout (Oncorhynchus mykiss) farms. Aquaculture, Vol. 289: 91-94

Klausen & Grønborg. 2010. Pilot scale testing of advanced oxidation processes for degradation of geosmin and MIB in recirculated aquaculture. Water Science & Technology p. 217-225.

Lee, S. & Radtke T. 1998. Exposure to formaldehyde among fish hatchery workers. *Appl. occup.environ. Hyg*., **13**, 3-6.

Møller, M. Arvin E. & Pedersen L. F. 2010. Degradation and effect of hydrogen peroxide in small-scale recirculation aquaculture system biofilters. Aquaculture Research, Vol. 41: 1113-1122

Noble, A.C. and S.T. Summerfelt. 1996. Diseases encountered in rainbow trout cultured in recirculating systems. *Annual Review of Fish Diseases* 6:65-92, 1996.

Pedersen,L.F., Pedersen,P.B., and Sortkjaer,O. 2007. Temperature-dependent and surface specific formaldehyde degradation in submerged biofilters. Aquacultural Engineering **36**: 127-136.

Pedersen, L.-F., Pedersen, P.B. Nielsen, J.L. & Nielsen, P.H. 2009. Peracetic acid degradation and effects on nitrification in recirculating aquaculture systems. Aquaculture. Vol. 296: 246-254.

Pedersen, L-F., C. Good & P. B. Pedersen. *In press.* Low-dose hydrogen peroxide application in closed recirculating aquaculture systems. North American Journal of Aquaculture

Pedersen, L-F. og Henriksen, N.H. 2011. Undersøgelse af vandbehandlingspraksis med brintoverilte og pereddikesyreprodukter på forskellige typer dambrug. [*Investigations of water treatment practices with H2O2 and peracetic acid at different fish farms- In Danish*]. Report, p 41. Accessed, June 2011 at http://www.danskakvakultur.dk/images/nh%20veterinær/Formalinsubstitution_Hovedrapport.pdf

Rach,J.J., Gaikowski,M.P., and Ramsay,R.T. 2000. Efficacy of hydrogen peroxide to control parasitic infestations on hatchery-reared fish. Journal of Aquatic Animal Health **12**: 267-273.

Rach,J.J. and Ramsay,R.T. 2000. Analytical verification of waterborne chemical treatment regimens in Hatchery raceways. North American Journal of Aquaculture **62**: 60-66.

Rach,J.J., Schreier,T.M., Howe,G.E., and Redman,S.D. 1997. Effect of species, life stage, and water temperature on the toxicity of hydrogen peroxide to fish. Progressive Fish-Culturist **59**: 41-46.

Rintamaki-Kinnunen,P., Rahkonen,M., Mannermaa-Keranen,A.L., Suomalainen,L.R., Mykra,H., and Valtonen,E.T. 2005. Treatment of ichthyophthiriasis after malachite green. I. Concrete tanks at salmonid farms. Diseases of Aquatic Organisms **64**: 69-76.

Saez,J.A. and Bowser,P.R. 2001. Hydrogen peroxide concentrations in hatchery culture units and effluent during and after treatment. North American Journal of Aquaculture **63**: 74-78.

Schmidt, L. J. Gaikowski M. P. & Gingerich W. H. 2006. Environmental assessment for the use of hydrogen peroxide in aquaculture for treating external fungal and bacterial diseases of cultured fish and fish eggs. USGS Report, 180 pages.

Schwartz, M.F., G.L. Bullock, J.A. Hankins, S.T. Summerfelt and J.A. Mathias. 2000. Effects of selected chemo- therapeutants on nitrification in fluidized-sand biofilters for coldwater fish production. *International Journal of Recirculating Aquaculture*. **1:** 61–81.

Sharrer MJ, Summerfelt ST, Bullock GL, Gleason LE, Taeuber J. 2005. Inactivation of bacteria using ultraviolet irradiation in a recirculating salmonid culture system. Aquacultural Engineering, Vol. 33(2): 135-149

Tanner,P.A. and Wong,A.Y.S. 1998. Spectrophotometric determination of hydrogen peroxide in rainwater. Analytica Chimica Acta **370**: 279-287.

Wagner, E. J., R. E. Arndt, E. J. Billman, A. Forest, and W. Cavender. 2008. Comparison of the efficacy of iodine, formalin, salt, and hydrogen peroxide for control of external bacteria on rainbow trout eggs. *North American Journal of Aquaculture* 70 (2):118-127.

Waldrop, T., Gearheart, M. and Good, C. 2009. Disinfecting recirculating aquaculture systems: Post harvest cleaning. Hatchery International, Jan/Feb. 38-39.

Figures (7) Tables (2)

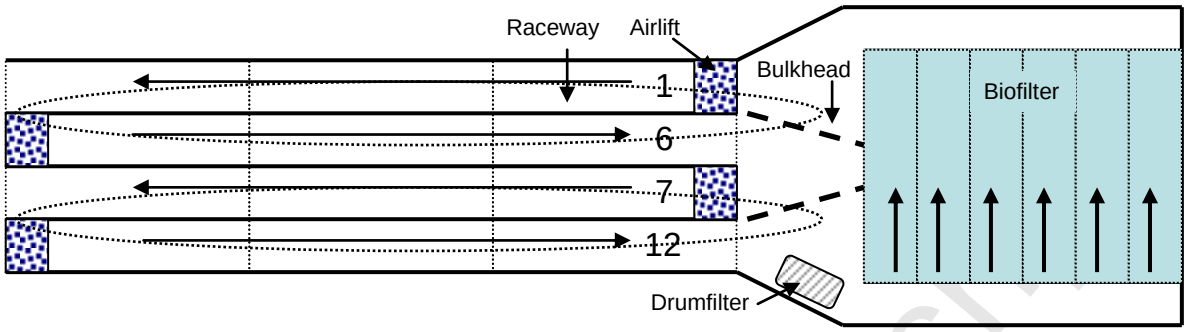

Fig.1. Schematics of the fish farm, with 6 biofilter section and 12 raceway rearing units (numbered). Long arrows show flow direction under normal operation; dotted lines indicate alternative flow pattern when biofilters are bypassed and the two sets of bulkheads are removed (not to scale).

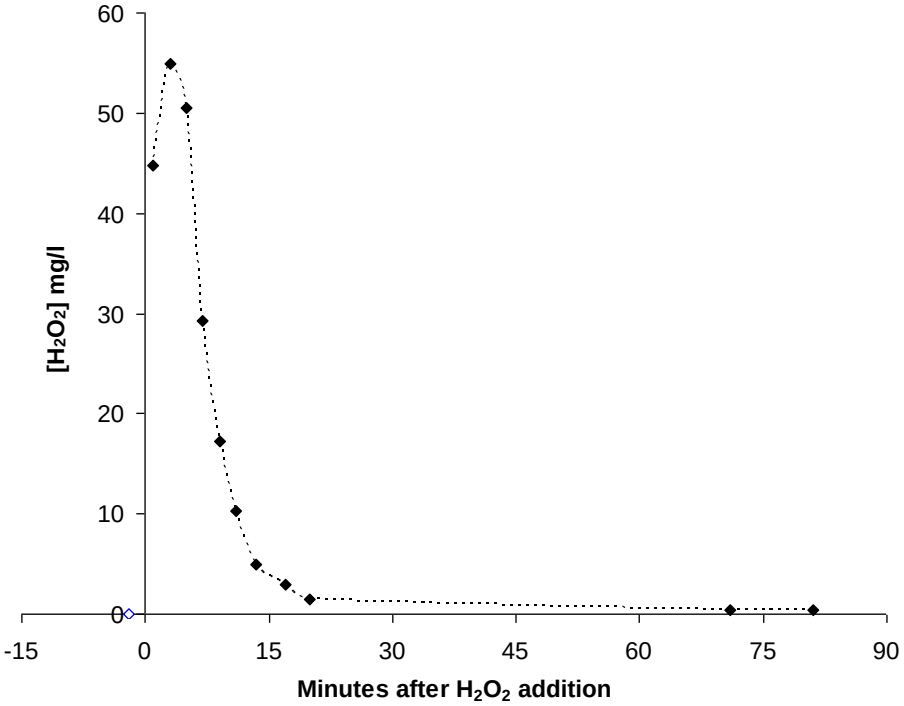

Fig.2. Concentration of hydrogen peroxide measured in the water of a 55 m^3 biofilter section exposed to 10 kg H_2O_2. Theoretical nominal H_2O_2 concentration was ~64 mg/l.

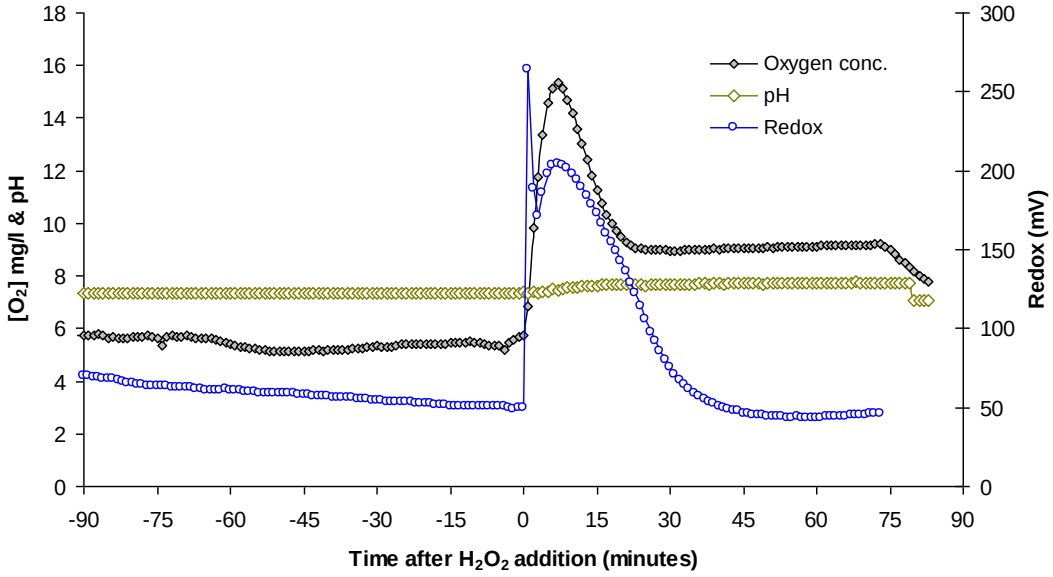

Fig.3. Logging data of oxygen, pH and Redox (ORP) from a trial where 10 kg 35% H_2O_2 was applied to a closed, disconnected biofilter section at t=0.

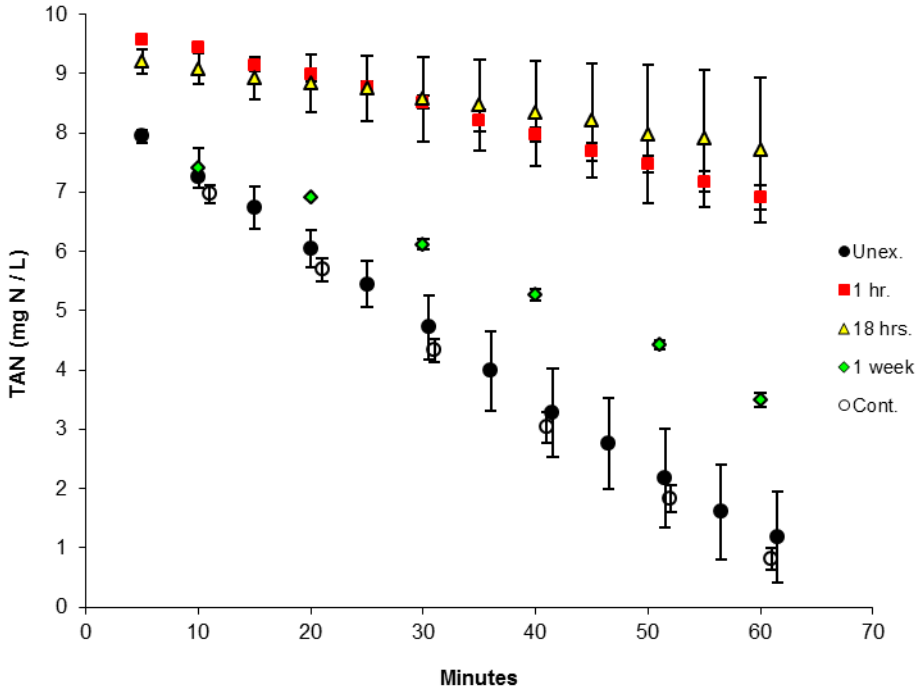

Fig.4. Removal of ammonia/ammonium (TAN concentration; mean± std. dev) from batch experiments with biofilter elements collected at Tingkærvad Trout farm. Experiments were made in a duplicates based on five sampling occasion: Biofilter elements were collected before H_2O_2 exposure (Unexposed), and again 1 hour, 18 hours and 1 week after H_2O_2 exposure. Biofilter elements from an identical biofilter section not exposed to H_2O_2 were collected at day 7(Cont.)

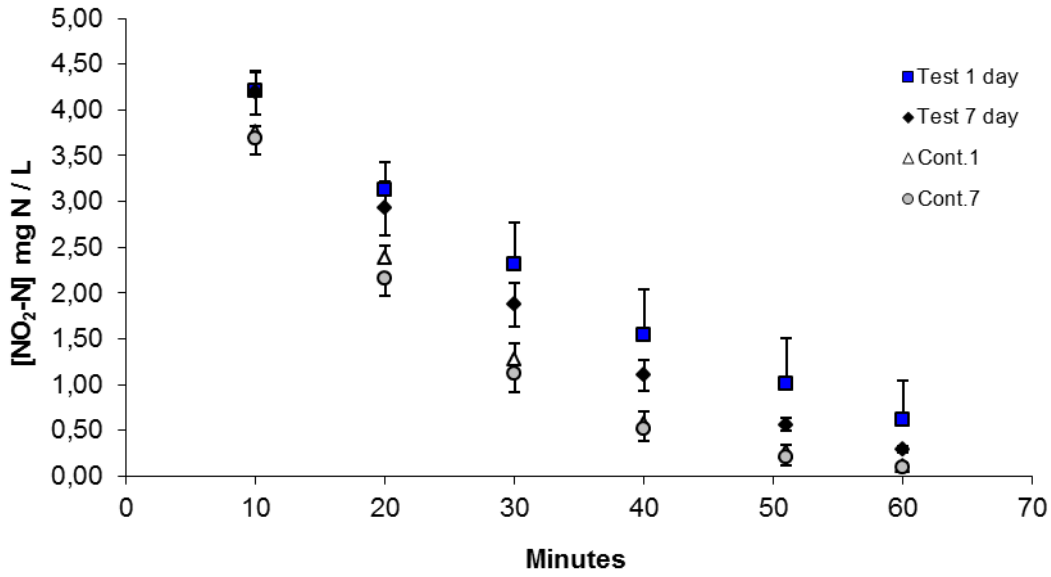

Fig.5. Nitrite-N concentration data (mean ± std. dev.) from batch experiments with biofilter elements. Experiments were made in a duplicate set-up with biofilter elements from two identical biofilter sections. One biofilter section was exposed to H_2O_2 (Test) whereas the other was unexposed (control). Experiments were made on two occasions (Day 1 and day 7 after exposure).

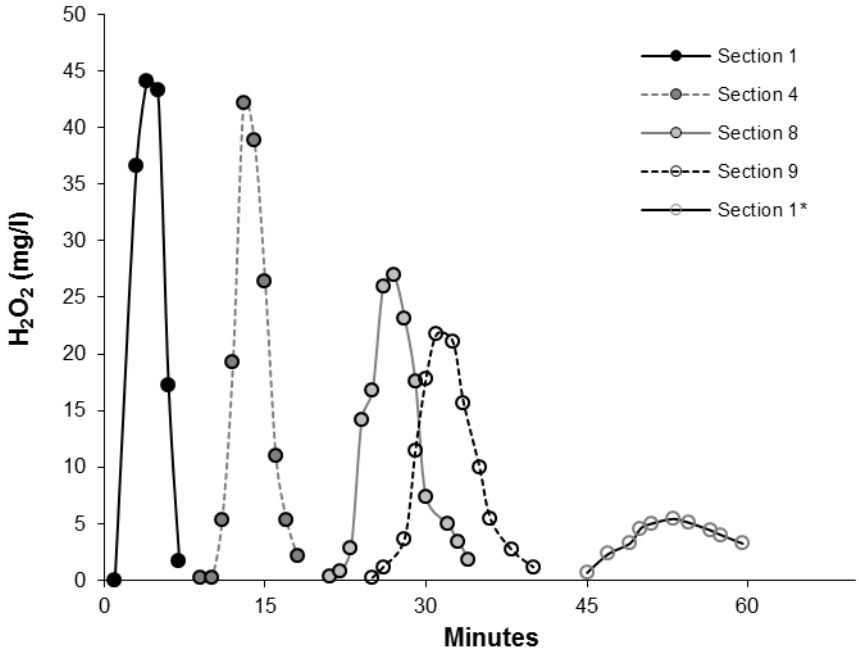

Fig.6. Concentration of H_2O_2 in the raceways after H_2O_2 addition at the inlet to raceway 1

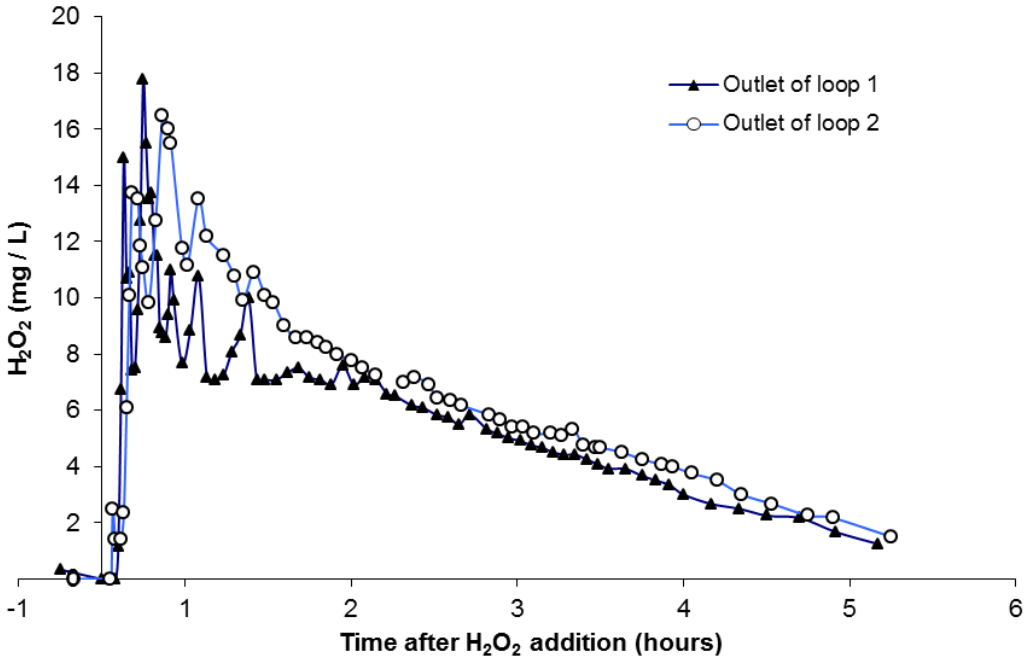

Fig.7. Concentration of hydrogen peroxide after addition of 4*20 L 35 % H_2O_2 to rearing units at Tingkærvad Trout farm. Loop 1 included raceway 1 to 6; loop 2 included raceway 7 to 12. Water samples were collected at two identical positions at the outlet from the two loops, sterile filtered, quenched and measured with a spectrophotometer. The nominal concentration equals 20 mg H_2O_2 /L assuming ideal mixing and no internal degradation.

Table 1: Fish farm data

Tingkjærvad Troutfarm	Specifications		Remarks
Rearing units (total)	1500	m^3	12 identical, serial units
Biofilter (total)	300	m^3	6 identical, parallel sections
Makeup flow (Q_m)	20	l/s	Ground water
Internal flow (Q_{reuse})	650	l/s	Circulated via airlift systems
Circulation time	50	min	
Biofilter characteristics[#]	100	l/s	Upflow
Filter volume (without media) V_0	60	m^3	Per biofilter section
Cross sectional area of filter A_{cross}	20	m^2	Per biofilter section
Filter volume (with media) V_F	50	m^3	Per biofilter section, adjusted for media and void space
Biofilter media characteristics			Combined double layer biofilter
Submerged upflow, fixed bed (lower layer)	14	m^3	BioBlok HD 150 (ExpoNet®); 150 m^2/m^3
Moving bed (upper layer)	14	m^3	Penta Plast; 800 m^2/m^3 according to manufacturer
Total active surface area of media (A_{media})	13300	m^2	

* Data on airlifts; sludge cones, drum filter etc. not included

\# Double layer compartment; data on air nozzles and void space below media layers are not provided

Table 2: Evaluation of biofilter performance measured in batch reactors with biofilter elements from Tingkærvad Trout Farm. Removal of total ammonia/ammonium nitrogen (TAN) were assessed in time series and calculated according to biofilter volumen and surface/volume specifications. Representative sub-samples of biofilter elements were taken out: *before* H_2O_2 application; at the *end* of the treatment period from the bypassed biofilters; and 1 hour *after* reopening into the biofilter section.

Test groups of biofilter elements	Max TAN removal (0°) g $N/m^2/d$
Before H_2O_2 addition	**0.69 ± 0,13**
End of treatment and before reopening the biofilter section	**0,71 ± 0,05**
One-hour after reopening the biofilter	**0,56 ± 0,12**